REVIEW

Probiotics for preterm infants?

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Infants nursed in special care baby units develop an abnormal pattern of microbial colonisation, which may contribute to disease. Enteric feeding of live microbial supplements (probiotics) may provide benefit to such infants and help to prevent diseases such as neonatal necrotising enterocolitis.

Although there is now a considerable body of experimental data and a burgeoning clinical trials literature, so far there have been few adequately controlled clinical trials of probiotics in preterm infants. There is a clear need for clinical trials of sufficient size to allow clinically important outcomes to be investigated.

Healthy infants develop a colonising microflora which is dominated in the bowel by non-pathogenic species such as bifidobacteria.[1] The early pattern of microbial colonisation probably contributes to normal development through a number of different pathways. These include enhancement of the mucosal protective barrier,[2 3] modification of systemic immune responses,[4–6] competitive exclusion of less desirable microbes,[7] protein and carbohydrate degradation, vitamin and butyrate production, and perhaps also mucosal differentiation.[8] The pattern of development of the bowel flora results from a complex interplay of nutritional, immunological, and environmental factors. It is generally accepted that the predominance of some bacteria such as bifidobacteria are beneficial and others such as enterobacteriaceae, *Pseudomonas aeruginosa*, and clostridia are detrimental.[9]

> "The abnormal pattern of colonisation in preterm infants may also contribute to the pathogenesis of neonatal necrotising enterocolitis"

Preterm infants in intensive care develop a very abnormal pattern of bowel colonisation compared with healthy infants and children.[10 11] Colonisation with many of the bacteria found in healthy breast fed infants is delayed.[2] Use of antibiotics, infection control procedures such as hand washing, reduced exposure to maternal microflora, and sterile feeds all act to limit the extent to which preterm infants are exposed to (and become colonised with) normal commensal microorganisms. The bowel becomes a major reservoir for antibiotic resistant bacteria, which can subsequently cause infection in the colonised infant and also spread to others in the intensive care unit. The abnormal pattern of colonisation in preterm infants may also contribute to the pathogenesis of neonatal necrotising enterocolitis (NEC).[12] The predominant facultative bacterial species in the faecal flora of preterm infants undergoing intensive care are staphylococci (coagulase negative staphylococcus spp and *Staphylococcus aureus*), enterobacteriaceae (such as *Klebsiella* spp), and enterococci. Clostridia are the most common anaerobes, with few if any infants showing colonisation with bifidobacteria—in contrast with healthy breast fed term infants in whom bifidobacteria predominate.[1] Yeasts may also be major components of the bowel flora of these infants.[13]

It is generally considered that diversity is an important factor in determining the stability of ecological systems to perturbation.[14] The faecal flora of a healthy adult may have more than 400 different culturable bacterial strains[15] compared with less than 20 in a preterm infant in intensive care.[10 11] It may be that the lack of microbial diversity in the bowel of preterm infants predisposes them to significant changes in patterns of colonisation such as the acquisition of antibiotic resistant strains or the loss of strains associated with antibiotic treatment.

> "Probiotic bacteria are defined as live microbial supplements that colonise the gut and provide benefit to the host."

One way to encourage bowel colonisation with "desirable" flora is through the administration of probiotic bacteria. Probiotic bacteria are defined as live microbial supplements that colonise the gut and provide benefit to the host.[16] There is increasing interest in the potential health benefits that may be derived from proactive management of bacterial colonisation of the gastrointestinal tract in preterm infants. The concept that the bacteria that live within us may be important determinants of health and disease was proposed by Metchnikov[17] and popularised by Douglas.[18] Since these early publications, an increasing body of scientific literature has lent credence to the view that bacterial flora modification may improve health. Many studies in both laboratory and farm animals suggest that probiotic feeding can provide benefits. Feeding of live microorganisms has led to developments in animal husbandry such as a reduction in the colonisation of chickens with *Salmonella enteritidis* phage type 4.[19–21]

The range of effects of probiotics on the gut are wide and include changes in intestinal permeability,[22–24] enhanced mucosal IgA responses,[25] increases in the production of anti-inflammatory cytokines,[25–29] and "normalisation" of gut microecology[30 31] (Box 1). There is an increasing body of evidence from clinical trials that feeding of live microbial supplements can

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provide nutritional and immunological benefits for humans, such as the prevention of recurrent *Clostridium difficile* infection and reduction in the duration of rotavirus diarrhoea.[32 33]

PROBIOTICS FOR PRETERM INFANTS?

Potential benefits from probiotic feeding for preterm infants include a reduction in the bowel reservoir of more pathogenic species, improved enteral nutrition, and reduced dependence on intravenous nutrition, an increased gut mucosal barrier to bacteria and bacterial products, and upregulation in protective immunity. Potentially, use of probiotics could lead to improvements in nutrition, a reduction in the incidence of sepsis and use of antibiotics, and prevention of neonatal NEC.

FEEDING IN THE PRETERM INFANT

The importance for the preterm baby of establishing enteral nutrition, preferably with breast milk, and of optimising early growth is established.[34] How this is most safely achieved in the group of babies at greatest risk of NEC and of infective episodes, those < 1000 g birth weight, is unclear. Apart from theoretical arguments arising from conflicting results of studies of the relation between enteral feeding and NEC,[35–37] there are practical problems in babies who may have poor gut motility and often tolerate enteral feeding only poorly. Clinicians adopt a number of approaches to the early management of this problem. These range from abandonment of milk feeds and total reliance on total parenteral nutrition, through minimal enteral nutrition—which may gain for the baby the physiological advantages of breast milk while avoiding a possible increased risk of NEC—to conventional use of breast milk in increasing volumes.

Any management strategy that safely brings forward the time at which full enteral nutrition can be achieved and central feeding lines can be removed would be of advantage to the neonatologist. The use of probiotics to promote food tolerance has been studied in other patients groups,[38] but there are few studies of the impact of probiotics on nutrition in preterm infants, although the human bowel flora itself has extensive metabolic activities.[9 39]

PREVENTION OF INFECTION IN PRETERM INFANTS

A high proportion of preterm infants (particularly those of very low birth weight) receiving intensive care suffer episodes of systemic infection with antibiotic resistant bacteria and fungi.[40] These infections further increase the risk of adverse outcomes such as chronic lung disease and brain injury.[40 41]

There are several mechanisms by which probiotic administration may be expected to reduce the incidence of infection in preterm infants: (*a*) an increased mucosal barrier to translocation of bacteria and bacterial products[24 42–49]; (*b*) a reduction in the incidence of suspected or proven neonatal NEC[50–54]; (*c*) improved enteral nutrition,[55] leading to a reduction in the use of intravenous feeding, which is a major risk factor for bacterial infection in hospital patients; (*d*) changes in the pattern of gastrointestinal tract colonisation, leading to a reduction in the extent to which preterm infants are colonised with potential pathogens such as enterococci[55] and possibly increased colonisation with desirable microflora such as *Streptococcus salivarius*[56]; (*e*) upregulation of immune responses.[57–62]

PREVENTION OF NEC

NEC is the most common acquired abdominal emergency in preterm infants receiving intensive care.[53] Known risk factors include enteral feeding and prematurity. The incidence of NEC and the severity increase with decreasing birth weight. Box 2 shows other factors that may contribute to the pathogenesis of NEC. Cases of NEC may cluster in time and place, and it has not been possible to reproduce the disease in germ free animals.[64] Furthermore, changes in bacterial metabolic activities precede the onset of NEC (such as the fermentation of carbohydrates to produce intramural gas (hydrogen)).[65] These observations have led to suggestions that bacteria contribute to the pathogenesis of NEC.

There is increasing interest and some evidence that probiotics may have a role in the control or prevention of inflammatory bowel disease.[65] Numerous clinical trials in children and adults have lent support to the view that probiotic administration can modify the severity of inflammatory bowel disease.[33 75 76] The range of organisms used has been very wide, from single widely characterised strains such as *Escherichia coli* strain Nissle, which has been used since 1917[77], to recent studies using complex mixtures of bacteria.[75]

As the scientific basis for the use of probiotics is established, it is likely that genetically engineered probiotics modified to produce specific properties will be increasingly used for the control of inflammatory bowel disease. Thus, Steidler and coworkers[78] showed that a genetically engineered probiotic bacterium delivering interleukin 10 reduced colitis in two different experimental mouse models. Box 3 shows potential mechanisms by which probiotics may prevent NEC. Enteral administration of probiotics has been shown to prevent NEC in a neonatal rat model using *Bifidobacterium infantis*.[53] The potential for use of probiotics in the treatment of established NEC remains unexplored.

STUDIES OF THE USE OF PROBIOTICS IN PRETERM INFANTS

There have been few clinical trials that have reported the outcomes for preterm infants given probiotics. Early comparative studies concentrated on the safety and colonisation potential of probiotics in this population and the impact of feeding probiotic bacteria on the enteric microflora of infants.[79 80] More recent studies have looked at different outcomes including NEC (see below),[42] enteral feed tolerance and weight gain,[55] and serum endotoxin levels.[47] Most of these studies have involved small numbers of infants nursed in a single neonatal intensive care unit. Placebo preparations have not been included in most studies; instead outcomes in infants given supplemented and unsupplemented feeds have been compared. One of the largest studies reported improved weight gain and food tolerance when *Bifidobacterium breve* strain BBG was given to preterm infants.[55] The antibody response in premature babies to oral administration of a probiotic *E coli* strain (Nissle 1917) was tested in a randomised placebo controlled blind trial. Specific *E coli* IgA antibodies were significantly higher in the test group, as was non-specific IgM.[81]

Infection and use of antibiotics was included as an outcome in an open randomised study[82] in which 50 infants received a bifidobacterium strain (*Topfer bifidus*) nasogastrically from day 3 to 21 and were compared with a control group. Those given bifidobacteria were more likely to be colonised with bifidobacteria than the control group, and received antibiotics for longer. However, in only one of 23 episodes of sepsis was a bifidobacterial predominance found on the day of onset of the episode, suggesting that bifidobacteria may confer a protective effect. In a recent multicentre double blind study from Italy, 585 infants of less than 33 weeks gestational age or birth weight less than 1500 g were randomised to receive *Lactobacillus rhamnosus* GG (6×10^9 colony forming units) once a day from the start of feeds to the time of discharge, or a placebo.[83] Outcome measures included the incidence of urinary tract infection, bacterial sepsis, and NEC. The numbers of babies with any of the three outcomes were surprisingly low, and there were no significant differences between the probiotic and placebo groups. The report gives no microbiological data on colonisation with *Lactobacillus* GG, and more than half of the infants received more than one course of antibiotic, so the extent to which infants were colonised and the relation between outcomes and colonisation cannot be examined. A "non-pathogenic" strain of *S aureus* (strain 502A) was used as

Box 1: Range of probiotic effects

- Reduced intestinal permeability
- Increased mucosal barrier to bacteria and bacterial products
- Upregulation of local and systemic immunity
- "Normalisation" of microflora
- Protection of mucosa from colonisation by pathogens
- Increased anti-inflammatory cytokines

Box 2: Factors that may be important in the pathogenesis of NEC

- Host susceptibility determinants
  - Levels of secretory IgM[66 67]
  - Levels of platelet activating factor (PAF) acetylhydrolase[68]
  - Hypoxic and/or ischaemic injury[69]
  - Enterocyte maturity[70]
- Presence of bacteria in gastrointestinal tract[12]
- Abnormal bacterial colonisation of gastrointestinal tract[10]
  - Propensity of enterobacteriaceae to translocate[71]
  - Lack of competing microflora[72]
  - Impact on mucosal differentiation[8]
- Antenatal steroids[10 73]
  - Mucosal maturation[63]
- Breast feeding[74]
  - Mucosal barrier[64]
  - PAF acetylhydrolase activity[68]

a spray to control outbreaks of staphylococcal infection in the USA during the 1960s. Over 4000 infants were colonised.[84] This procedure was associated with control of a number of nursery outbreaks of staphylococcal infection. Periumbilical infection was described in a number of infants,[85] and one infant suffered a serious adverse event.[86]

There have been no randomised, placebo controlled, blinded studies of probiotics of sufficient size to determine the impact of probiotic feeding on incidence of NEC. In an open study from South America, a reduction in the incidence of NEC was reported in infants in a neonatal intensive care unit after the introduction of enteral feeding with *Lactobacillus acidophilus* and *B infantis* by comparison with historical controls.[52]

THE FUTURE

Many different species of bacteria and fungi have been used as probiotics. Even within a species such as *E coli* or *S aureus*, there are probiotic and pathogenic strains, so the range of potential probiotics is enormous. Currently we do not know which microbial characteristics are desirable for particular groups of patients. Selection of strains for use in clinical trials is based on microbial characteristics such as ability to survive gastric acid and colonise the gut, production of factors that inhibit the growth of pathogenic bacteria (such as H_2O_2 by lactobacilli), and other desirable (generally metabolic or immunological) effects. Often, but not always, strains that are selected for human study have been extensively studied in laboratory animals. It is generally considered that a predominance of bifidobacteria in the gut of infants is desirable, but we do not know if the primary objective is to inhibit colonisation of the gut by undesirable pathogens or to promote colonisation with "healthy" bacteria, or both.

Nor do we know how to optimise colonisation with probiotics. Prebiotics, such as oligofructose for bifidobacteria, encourage proliferation of specific bacteria in the gut.[87] Prebiotics can be used by themselves or in combination with probiotics. Probiotics tend to be selected for antibiotic susceptibility, but, in patient populations in which antibiotics are often used, perhaps the choice and mode of administration of antibiotics will have to be modified to facilitate probiotic colonisation.

Box 3: Potential mechanisms by which probiotics may prevent NEC

- Reduced mucosal colonisation by potential pathogens
- Increased barrier to translocation of bacteria and bacterial products across mucosa
- Competitive exclusion of potential pathogens
- Modification of host response to microbial products
- Enhancing enteral nutrition

Currently many different preparations containing live probiotic bacteria are available as foodstuffs from commercial outlets and are being given to patients both within and without the context of clinical trials. Probiotics are considered to be foods and are not subject to the stringent controls that are applied to licensed drugs. There is an increasing trend for probiotics to be administered to a variety of patient groups including preterm infants for a variety of reasons. Currently there is a paucity of data on their safety or efficacy in preterm infants.

CONCLUSION

"Normalisation" of the bowel flora of preterm infants to make it more like that of a healthy breast fed infant may or may not produce clinical benefits. There are few published safety data on probiotic use in preterm infants. The ideal characteristics of probiotic strains to be used in preterm infants have yet to be defined. Probiotics may offer potential benefits for preterm infants, but there is a need for clinical trials of sufficient size to allow clinically important outcomes to be investigated.

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